Remarks

Claims 1-13 are currently pending in the application. In order to advance prosecution, Applicants have canceled claim 1, amended claims 2-13, and added claims 14-16. The amendments to the pending claims were made to clarify the scope of coverage and to more particularly point out and distinctly claim the present invention.

The amendments to the pending claims are made without prejudice, do not constitute amendments to overcome any prior art rejections under 35 U.S.C. §§ 102 or 103, and are fully supported by the specification as filed. For example, support for the phrase "average optical density of stained AKT protein per pixel of cellular area" appears, *inter alia*, at page 10, lines 16-30. Further support for all the claim amendments can be found throughout the specification.

Cancellation of claim 1 is without prejudice or disclaimer, and Applicants make no admission regarding the patentability of this subject matter and should not be so construed. Applicants reserve the right to pursue this subject matter in this or in any other appropriate patent application.

Discussion of the Specification Informalities

The specification was objected to for the use of the terms AKT and PTEN because the full name or explanation of the above abbreviations is required when they appear in the specification for the first time. However, Applicants respectfully point out that contrary to the Office Action's assertion, the terms "AKT" and "PTEN" are not abbreviations, but rather are the non-abbreviated names of specific cellular proteins. For example, AKT is the name used for cellular proteins that are "involved in mediating avoidance of apoptosis in tumor cells." *Specification* at page 4, lines 30-33. In addition, PTEN is a human tumor suppressor whose loss

correlates with increased AKT activity. *Id.* at page 5, lines 6-7. In fact, the acceptance by those skilled in the art of the name AKT as it relates to a cellular protein capable of inhibiting apoptosis and PTEN as it relates to a tumor suppressor is evident from the Liu reference (Liu *et al.* (Biochemical and Biophysical Research Communications, 1999, vol. 261, pp. 897-903)) cited by the Examiner in support of the 35 U.S.C. § 103 rejections.

In addition, the Examiner noted that the Application contains the use of trademarks, but acknowledged that trademarks are permissible in patent applications, although the proprietary nature of the marks should be respected. Correspondingly, the specification has been amended to comply with the Examiner's request. However, Applicants make no admission regarding the validity or continued existence of the trademarks as indicated by the Examiner. No new matter has been introduced by any of these amendments.

Discussion of the Double Patenting Rejection

The Office Action provisionally rejected claims 1, 2, 8, 10, and 12 under the judicially created doctrine of obviousness-type double patenting as being allegedly unpatentable over claims 1-7, 10, and 14 of copending Application No. 09/760,120. Applicants will consider submitting a terminal disclaimer when the claims are otherwise in condition for allowance.

Discussion of the 35 U.S.C. § 112, Second Paragraph Rejection

Claims 1-13 are rejected under 35 U.S.C. § 112, ¶ 2, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicants regard as the invention. The rejection is respectively traversed.

With respect to the rejection of the claims based on the use of the term AKT, the Office Action stated that the recitation of AKT is indefinite because the full name or explanation of abbreviations are required when they appear in the claims for the first time. However, as stated above, Applicants respectfully point out that "AKT" is not an abbreviation, but rather the non-abbreviated name used for cellular proteins that are "involved in mediating avoidance of apoptosis in tumor cells." *Specification* at page 4, lines 30-31. In fact, as previously stated, the acceptance by those skilled in the art of the name AKT as it relates to a cellular protein capable of inhibiting apoptosis is evident from the Liu reference (Liu *et al.* (Biochemical and Biophysical Research Communications, 1999, vol. 261, pp. 897-903)) cited by the Examiner in support of the 35 U.S.C. § 103 rejections. Therefore the term is not indefinite as recited in the claim. Accordingly, Applicants respectfully request withdrawal of this rejection and requests reconsideration of the claims.

With respect to claim 1, the Office Action stated that it is not clear what applicants intend in the recitation of "an optical density of staining." Although not acquiescing to this ground of rejection, Applicants have amended the claims, including deleting claim 1 and adding claim 14 that does not contain this limitation, to better clarify the invention. Applicants respectfully contend that the claim amendments have overcome the asserted ground of rejection.

Discussion of the 35 U.S.C. § 103(a) Rejection

Claims 1-13 are rejected under 35 U.S.C. § 103(a) as being obvious over Bacus (U.S. 5,288,477) ("Bacus") in view of Liu *et al.* (Biochemical and Biophysical Research Communications, 1999, vol. 261, pp. 897-903) ("Liu") and further in view of Slamon *et al.* (U.S. 5,846,749) ("Slamon"). Applicants respectfully traverse this rejection.

An analysis for obviousness requires a determination of the scope and content of the prior art, the differences between the prior art and the claims at issue must be ascertained, and the level of ordinary skill in the pertinent art must be resolved. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). To establish a *prima facie* case of obviousness, the Office must show three basic criteria: (1) there must be a suggestion or motivation to combine the reference teachings; (2) there must be a reasonable expectation of success; and (3) all of the claimed limitations must be taught or suggested in the combined prior art references. M.P.E.P. § 2143.

The instantly claimed invention is directed towards methods for determining the quantity of AKT protein, the AKT protein activation level or both in cells of a biological sample. This method requires, among other things, that the average optical density of stained AKT protein per pixel of cellular area be determined by, for example, image analysis. Because the average optical density of stained protein *per pixel* of cellular area is detected, the actual number of cells present in the image field is irrelevant and never need be actually determined.

None of the cited references, alone or in combination, teach or suggest the instantly claimed method. Bacus teaches a method for prognosticating the effectiveness of a therapeutic agent in the treatment of a cancer by measuring the ability of the therapeutic agent to induce terminal differentiation wherein malignant cells of the cancer overexpress an oncogene product. In Bacus, the effectiveness of the therapeutic agent is determined by comparing the *percentage of cells* that exhibit markers of terminal differentiation in a first portion of a biopsy that was treated with the therapeutic agent to the *percentage of cells* that exhibit markers of terminal differentiation in a second portion of a biopsy that was not treated with the therapeutic agent. In fact, Bacus teaches that it is preferable to determine "the average amount of membrane-bound

HER-2/*neu per cell* . . . in obtaining cell percentages." *Bacus*, column 8, lines 50-52 (emphasis added).

Bacus, however, does not teach a method of determining the average optical density of stained AKT protein per pixel of cellular area. Instead, Bacus teaches the quantitation of a protein such as HER-2/*neu* by determining the signal value for a *known* number of cells. Thus, according the Bacus, the signal value *per cell* is determined, rather that the average optical density of stained AKT protein *per pixel* of cellular area. As an example, Bacus teaches that a DNA stain can be used to quantify the amount of HER-2/*neu* per cell, because the DNA stain can be used determine the amount of cells in a preparation, and "the sum of optical density for the protein is then divided by this number of cells to yield the average protein content *per cell*." *Bacus*, column 10, lines 38-65 (emphasis added). Most important, however, is the fact that Bacus does not teach how to quantitate cellular proteins *without* knowing or determining the number of cells that are immunostained. Moreover, Bacus does not teach how to determine the average optical density of stained AKT protein per pixel of cellular area, let alone how to use that information to determine the quantity of AKT in cells of a biological sample. As a consequence, Bacus does not teach or suggest the presently claimed invention.

The deficiencies of Bacus are not overcome by the combination with the other cited art. Liu describes that Heregulin is a potent and rapid activator of AKT in breast cancer cells. *See Liu*, Abstract. The Office Action cites Liu for proposition that detection of AKT had previously been performed in breast cancer cells. The Office Action further cites Liu for the proportion that AKT can be either AKT1 or AKT2, and that four different breast cancer cell lines were used to detect AKT. However, Liu only teaches the detection of AKT by either Western blot analysis or immunoprecipitation followed by an assay of AKT enzymatic activity. Indeed, Liu does not

teach detection of AKT through the use of image analysis, and certainly does not teach, much less suggest a method of determining the average optical density of stained AKT protein per pixel of cellular area. Therefore, Liu does not teach, nor does it suggest, the presently claimed invention.

Slamon is cited by the Office Action as teaching that a wide variety of assays can be used to detect and quantitate tissue proteins. For example, the Office Action cites Slamon as teaching immunoassays, including ELISA, immunohistochemical staining, Northern hybridization, and measuring mRNA. In addition, Slamon is cited as teaching the production of a calibration curve and calculation of determined values by comparison with the calibration curve.

However, Slamon does not teach how to determine the average optical density of stained AKT protein per pixel of cellular area. In fact, in reference to the presently claimed invention, Slamon deficiencies are similar to the deficiencies found in Bacus. Slamon quatitates surface membrane and cytosolic proteins by determining the signal value from a *known* number of cells, and relates this value to values obtained with control cells. Thus, according to Slamon, the signal value *per cell* is determined, rather that the average optical density of stained target protein *per pixel* of cellular area. As an example, all of the independent method claims of the Slamon patent require "determining the signal value *from a known number of said fixed cells* by computerized image analysis." *See, e.g.*, *Slamon*, column 16, lines 12-14 (emphesis added). As a further example, the Slamon specification provides for measurement of protein immunostaining, specifically HER2/*neu*, and measurement of DNA content in individual tumor cells. *See, e.g.*, *id.* at column 10, lines 52-57. The Slamon reference does not, however, teach how to quantitate cellular proteins using image analysis *without* knowing or determining the number of cells that are immunostained. Moreover, Slamon does not teach, much less suggest, how to determine the

average optical density of stained AKT protein per pixel of cellular area, let alone how to use that information to determine the quantity of AKT protein in cells of a biological sample. As a consequence, Slamon does not teach, much less suggest, the presently claimed invention.

Thus, Applicants respectfully contend that the Office Action has failed to establish a *prima facie* case of obviousness because first, there is no teaching, suggestion or motivation to combine the cited reference, and second, even if the references are improperly combined, all of claim limitations are not taught or suggested by either Bacus, Liu, or Slamon. The Office Action argues that it would have been obvious to determine the AKT protein expression in a cell or tissue sample by means of staining one portion of the sample with a detectably labeled antibody and quantitating the amount of AKT protein by comparing the optical density of the samples with a calibration curve. Further, the Office Action argues that a skilled artisan would have been motivated to do so with a reasonable expectation of success by the teachings of Bacus on the measuring of a breast cancer related protein marker by quantification of HER2/*neu* protein by selecting optical density summation analysis in conjunction with staining procedure.

However, the mere description in Bacus of the use of image analysis to quantitate a protein such as HER-2/*neu* by determining the signal value for a *known* number of cells does not amount to a teaching or suggestion to determine the average optical density of stained AKT protein per pixel of cellular area, much less how to use this information in the claimed methods for determining the quantity of AKT protein, the AKT protein activation level or both in cells of a biological sample. The secondary references do not cure this infirmity. Liu is merely concerned with studying the role of AKT in the heregulin pathway to help understand the pathogenesis of cancer. Slamon is concerned with the quantitation of proteins *per cell*. None of these references contemplate or teach methods for determining the average optical density of

stained AKT protein per pixel of cellular area. *A fortiori*, the references certainly do not teach or even contemplate the instantly-claimed methods of determining the quantity of AKT protein, the AKT protein activation level or both in cells of a biological sample by, *inter alia*, determining the average optical density of stained AKT protein per pixel of cellular area. In the absence of such teaching, Applicants contend there was simply no motivation to combine these references as the Office Action suggests.

Applicants respectfully submit that the Office Action has engaged in impermissible hindsight to support its argument. In this regard, the Federal Circuit dictates, "[i]t is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious. This court has previously stated that '[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." *In re Fritch*, 23 U.S.P.Q.2d 1780, 1784 (Fed Cir. 1992) (citations omitted) (quoting *In re Fine*, 5 U.S.P.Q.2d 1596, 1600 (Fed. Cir. 1988)); *see also Para-Ordnance Mfg., Inc. v. SGS Imposters Int'l Inc.*, 37 U.S.P.Q. 1237, 1239 (Fed. Cir. 1995) ("Obviousness may not be established using hindsight or in view of the teachings or suggestions of the inventor."); *Heidelberger Druckmaschinen AG v. Hantscho Commercial Prods. Inc.*, 30 U.S.P.Q.2d 1377 (Fed. Cir. 1993) ("The motivation to combine references cannot come from the invention itself."); *Interconnect Planning Corp. v. Feil*, 227 U.S.P.Q. 543, 547 (Fed. Cir. 1985) ("The invention must be viewed not with the blueprint drawn by the inventor, but in the state of the art that existed at the time."). Applicants respectfully submit that what the law precludes is precisely the basis for the asserted obviousness rejection.

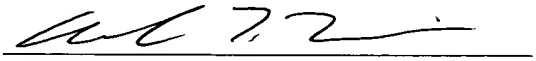
For the reasons set forth above, none of the references cited in support of this ground of rejection, Bacus, Liu, or Slamon, taken either alone or in any combination, disclose, either individually or in combination, a method for determining the quantity of AKT protein, the AKT protein activation level or both in cells of a biological sample by, *inter alia*, determining the average optical density of stained AKT protein per pixel of cellular area. Accordingly, Applicants respectfully request withdrawal of this rejection and requests reconsideration of the claims.

Conclusion

In view of the above amendments and remarks, the application is considered to be in good and proper form for allowance and the Examiner is respectfully requested to pass this application to issue. If there are any questions or comments regarding this Response or application, the Examiner is encouraged to contact the undersigned attorney as indicated below.

Respectfully Submitted,

Date: January 21, 2004



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